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6-Trifluoromethyl Pyridoxine: Novel ^{19}F -NMR pH Indicator for *In Vivo* Detection

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Abstract

pH plays an important role in tumor proliferation, angiogenesis, metabolic control and the efficacy of cytotoxic therapy, and accurate non-invasive assessment of tumor pH promises to provide insight into developmental processes and prognostic information. In this paper, we report the design, synthesis and characterization of two novel pH indicators 6-trifluoromethyl pyridoxine **8** and α^4, α^5 -di-*O*-[3'-*O*-(β -*D*-glucopyranosyl)-propyl]-6-trifluoromethyl pyridoxine **17**, and demonstrate **8** as an extracellular ^{19}F -NMR pH probe to assess pH_e of various tumors *in vivo*.

Keywords

pH indicator; synthesis; characterization; ^{19}F -NMR; signal enhancement; tumor *in vivo* pH detection

INTRODUCTION

The pH gradient between the interstitial and intracellular compartments is involved in many cell regulatory processes, and strongly influences drug uptake.^[1] Tumor pH also influences cell thermosensitivity, radiation sensitivity, proliferation and the efficacy of cancer therapy.^[2,3] The accurate non-invasive assessment of tumor pH promises to provide insight into the developmental process and prognostic information regarding therapeutic outcome. Previously, we demonstrated that 6-fluoropyridoxine (FPOL, Figure 1) can be used to measure both intra- and extracellular pH simultaneously providing exceptional sensitivity to pH changes in whole blood and the perfused rat heart.^[1a,b,4–6] However, its $\text{p}K_\text{a}$ =8.2 is not ideal for measurements under normal physiological conditions (pH 6.5~7.5). As a continuing work we report herein another strategy through introduction of a trifluoromethyl (CF_3 -) group instead of a fluorine atom at the 6-position of vitamin B₆ with the aims of modifying the $\text{p}K_\text{a}$ to physiological conditions and raising ^{19}F -signal to noise ratio.

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Notes

The authors declare no competing financial interest.

RESULTS AND DISCUSSION

Design and Synthesis

The introduction of a trifluoromethyl (CF_3) group into an organic compound can bring about remarkable changes in its physical, chemical and biological properties, making them suitable for diverse applications in pharmaceuticals and agrochemistry.^[7,8] We demonstrated that the introduction of CF_3 group in place of fluorine atom in phenols can enhance ^{19}F -NMR signal and modify the pK_a value ideal.^[9,10]

A wide variety of methods have been developed for introducing a CF_3 group into organic compounds,^[11] with (trifluoromethyl)trimethylsilane (Me_3SiCF_3) as a nucleophilic trifluoromethylating reagent becoming the method of choice.^[12,13] In this study we demonstrate a strategy that utilizes the iodination derivative **3** of vitamin B_6 (**1**) to react with Me_3SiCF_3 for synthesis of target compound 6-trifluoromethyl pyridoxine **8** (Figure 2).

A three-step procedure for halogenation of vitamin B_6 via 6-aminopyridoxine has been reported for the synthesis of the ^{19}F -NMR pH indicator 6-fluoropyridoxine by the modified Schiemann reaction, resulting in ~28% overall yield.^[1,6,14,15] We have now developed a more effective and direct method for obtaining the key intermediate 6-iodopyridoxine (**2**) in high yield. Reaction of **1** with iodine in 10% aqueous K_2CO_3 solution and avoiding light afforded **2** in 73% yield. For convenient blocking and de-protection, we initially proposed the acetylation as protecting strategy. Acetylation of **2** as usual work up gave 3, α^4 , α^5 -tri-*O*-acetyl-6-iodopyridoxine (**3**) in high yield (93%). However, trifluoromethylation of **3** with Me_3SiCF_3 gave the desired compound 3, α^4 , α^5 -tri-*O*-acetyl-6-trifluoromethyl pyridoxine (**4**) in only 40% yield. Further purification separated 12% of α^4 , α^5 -di-*O*-acetyl-6-trifluoromethyl pyridoxine (**5**). Presumably this resulted from the introduction of the highly electron-withdrawing 6-trifluoromethyl group in **4** that makes the *para* Ac- O_3 bond much polarized and activates its C=O group, which competed against the C₆-I bond in **3** to consume the trifluoromethylation reagent Me_3SiCF_3 . The proposed mechanism is depicted in Scheme 1.

To improve the process we employed benzyl ether as an alternative protecting group for 3, α^4 , α^5 -hydroxyl. Benzylation of **2** with benzyl bromide afforded 3, α^4 , α^5 -tri-*O*-benzyl-6-iodopyridoxine (**6**) in quantitative yield, which reacted with Me_3SiCF_3 in a similar procedure for the preparation of **4**, giving 3, α^4 , α^5 -tri-*O*-benzyl-6-trifluoromethyl pyridoxine (**7**) in excellent yield (96%). Overnight hydrogenation of **7** in ethanolic solution with the catalyst of 5% Pd/C provided the partial debenzylation product α^4 , α^5 -di-*O*-benzyl-6-trifluoromethyl pyridoxine (**9**) in quantitative yield. However, the α^4 , α^5 -di-*O*-benzyl groups could not be removed even with extended reaction times up to 1 week. Testing various acids as co-solvents and catalysts showed that anhydrous AcOH-EtOH (1:10 V/V') allowed stepwise cleavage of the 3, α^5 -di-*O*-benzyl groups in **7** yielding **10** in 1 day, then the α^4 -*O*-benzyl group in another day resulting in **8** with total yield of 100%. As expected, 6-trifluoromethyl pyridoxine **8** yielded higher signal to noise than 6-fluoropyridoxine (FPOL) and its derivatives.^[1a,b] Importantly, it has an ideal pK_a =6.83, but with much less ^{19}F NMR sensitivity to pH ($\Delta\delta$ =1.61ppm) and poorer water solubility (FPOL: 17.8mg/mL, **8**: 5.8mg/mL in H_2O at room temperature).

We found that modification of 4- and/or 5-methylenedihydroxyl moieties of 6-fluoropyridoxine resulted in changes of the pK_a and ^{19}F chemical shifts.^[1a,b] Previously, we successfully enhanced the solubility of ^{19}F -NMR and ^1H -MRI β -galactosidase reporters by conjugating them with carbohydrates.^[16] Prompted by these results, we designed another novel molecule **17** with two *D*-glucoses coupled to the 4- and 5-methylenedihydroxyl moieties of 6-trifluoromethyl pyridoxine **8** (Figure 3).

Starting with **2** as the initial molecule, the primary challenge was the regioselective protection among its three hydroxyl groups. However, pK_a calculations using the advanced chemistry development software (www.acdlabs.com) indicated the 3-phenolic hydroxyl ($pK_a=9.27\pm0.10$) is much more acidic than 4- and 5-methylenedioxy ($pK_a=13.31\pm0.10$ and 13.81 ± 0.10 , respectively), which suggests that phase-transfer-catalysis at pH=9~10 could provide regioselective protection of the 3-phenolic hydroxyl. To the well-stirred mixture of **2** in bi-phasic $\text{CH}_2\text{Cl}_2\text{-H}_2\text{O}$ (pH=9~10) at room temperature which was catalyzed by tetrabutyl-ammonium bromide (TBAB), benzyl bromide (1.1 equiv.) was added dropwise over a period of 4~5h. 3-*O*-benzyl-6-iodopyridoxine **11** was isolated as a major product in 74% yield. Its stereochemistry was confirmed by its $^1\text{H-NMR}$ spectrum where $\alpha^4, \alpha^5\text{-CH}_2$ exhibit doublets with coupling constants of $J_{\text{H-4,HO-4}}=5.6\text{Hz}$ at 4.87ppm, and $J_{\text{H-5,HO-5}}=6.4\text{Hz}$ at 4.73ppm. Treatment of **11** with an excess of allyl bromide gave 3-*O*-benzyl- α^4, α^5 -di-*O*-allyl-6-iodopyridoxine **12** in quantitative yield. It was then subjected to the procedure described for the preparation of **4** and **7** giving 3-*O*-benzyl- α^4, α^5 -di-*O*-allyl-6-trifluoromethyl pyridoxine **13** in an excellent yield (92%). The diallylated derivative **13** proceeded regioselective hydroboration by using 9-borabicyclo-[3.3.1] nonane (9-BBN), and subsequent alkaline oxidation with H_2O_2 giving 3-*O*-benzyl- α^4, α^5 -di-*O*-(3-hydroxypropyl)-6-trifluoromethyl pyridoxine **14** in 86% yield.

Condensation of **14** with 2, 3, 4, 6-tetra-*O*-acetyl- β -*D*-glucopyranosyl bromide accomplished 3-*O*-benzyl- α^4, α^5 -di-*O*-[3'-*O*-(2, 3, 4, 6-tetra-*O*-acetyl- β -*D*-glucopyranosyl)-propyl]-6-trifluoromethyl pyridoxine **15** in 88% yield. The ESI-MS displayed the expected molecular ion at m/z 1103 and quasimolecular ion at m/z 1104 $[\text{M}+\text{H}]^+$, corresponding to the fully adorned derivative with two 2, 3, 4, 6-tetra-*O*-acetyl- β -*D*-glucopyranosyl units. The identity of **15** was established using ^1H and $^{13}\text{C-NMR}$ based on the anomeric protons H-1' and H-1'' of *D*-glucoses at 4.48 and 4.41ppm, respectively, with two well resolved doublets $J_{1',2'}=J_{1'',2''}\sim 8.0\text{Hz}$ and $J_{2',3'}=J_{2'',3''}\sim 10\text{Hz}$, which confirmed both *D*-glucoses in the β -configuration with 4C_1 chair conformation, whereas the anomeric carbons C-1' and C-1'' at 101.02 ppm are in accordance.

Compound **15** was deacetylated with NH_3/MeOH from 0°C to room temperature giving 3-*O*-benzyl- α^4, α^5 -di-*O*-[3'-*O*-(β -*D*-glucopyranosyl)-propyl]-6-trifluoromethyl pyridoxine **16** in quantitative yield, and followed by hydrogenation catalyzed with 5% Pd/C overnight affording quantitative yield of target compound α^4, α^5 -di-*O*-[3'-*O*-(β -*D*-glucopyranosyl)-propyl]-6-trifluoromethyl pyridoxine **17** with an overall yield of 52%.

Characterization as $^{19}\text{F-NMR}$ pH Indicator

The ^{19}F chemical shifts upon pH changes (pH electrode) were measured with respect to sodium trifluoroacetate (NaTFA , $\delta_{\text{F}}=0\text{ppm}$). Both **8** and **17** exhibited single narrow $^{19}\text{F-NMR}$ signals in 0.9% saline or PBS essentially invariant ($\Delta\delta$ 0.03ppm) with temperatures ranging from 25 to 37°C . Figure 4 shows the titration curves of **8** and **17** in saline between pH 2~13 at 25°C . From the titration curves their pK_a , $\delta_{(\text{acid})}$ and $\delta_{(\text{base})}$ were determined from the Henderson-Hasselbach equation^[1a,b] (Table 1).

Given that both **8** and **17** show the feasibility as $^{19}\text{F-NMR}$ pH indicators, **8** has a more ideal pK_a for sampling biological system *in vivo*, making it favored for further evaluation.

$^{19}\text{F-NMR}$ pH Assessment in Perfused Heart and Whole Blood

The $^{19}\text{F-NMR}$ spectrum (376 MHz) of **8** in Langendorff perfused rat heart exhibited only a single ^{19}F -resonance at $\delta_{\text{F}}=16.41\text{ppm}$ corresponding to pH=7.39 (Figure 5a). The intra- and extracellular inorganic phosphates of Langendorff perfused rat heart was sampled by $^{31}\text{P-NMR}$, which provided the intracellular $\text{pH}_i=7.04$ ($\delta_{\text{Pi}}=4.88\text{ppm}$) and extracellular $\text{pH}_e=7.42$

($\delta_{\text{pe}}=5.32\text{ppm}$).^[1a,b] When the ^{19}F -NMR of **8** is compared to these results it becomes evident that **8** does not enter cells and reports only the extracellular pH_e . This was confirmed by the ^{19}F -NMR spectrum of **8** in whole rabbit blood which showed a single ^{19}F peak at $\delta_{\text{F}}=16.52\text{ppm}$ ($\text{pH}_\text{e}=7.44$) (Figure 5b).

In vivo ^{19}F -NMR pH Measurements in Tumors

Extracellular pH_e in human tumors has been shown to be associated with tumorigenic transformation, chromosomal rearrangements, induction of growth factors and proteases, extracellular matrix breakdown, and increased migration and invasion.^[2c,e] To evaluate the efficacy of this novel ^{19}F -NMR pH_e reporter molecule for sampling the tumor microenvironment, it was directly injected into rats bearing pedicle tumors. Firstly, pH_e reporter **8** (320mg/kg) and NaTFA (200mg/kg) in DMSO/saline (1:3 V/V') were injected i.p. into an anesthetized (with isoflurane) Copenhagen rat bearing a Dunning R3327-AT1 prostate tumor (tumor size: 2.4cm $\times$ 3.1cm $\times$ 1.8cm). **8** was detected by ^{19}F -NMR in the tumor 30 minutes after injection. The single broad ^{19}F -signal centered at $\delta_{\text{F}}=16.28\text{ppm}$ indicated tumor heterogeneity with mean $\text{pH}_\text{e}=7.20$.^[1b]

Similarly, **8** was detected in a 13762NF rat mammary tumor (tumor size: 1.8cm $\times$ 1.0cm $\times$ 2.0cm) after 8 minutes following an i.p. injection of the same doses into a Fisher 344 rat. By comparing the ^{19}F -NMR spectral intensities it is estimated that approximately 90% of **8** was in the 13762NF tumor. A broad single ^{19}F -resonance was obtained at $\delta_{\text{F}}=16.23\text{ppm}$ ($\text{pH}_\text{e}=7.14$) with biological clearance over 100 minutes (Figure 6A). This pH value was commensurate with micro electrode measurements in three Fisher 344 rats bearing 13762NF breast tumors various in 3.2, 7.5 and 9.2cm³, showing the most frequent $\text{pH}_\text{e}=7.03$ (Figure 6B).

Encouraged by these *in vivo* measurements of tumor pH_e , we also investigated 6-trifluoromethyl pyridoxine **8** in other animal models. After i.p. injection of **8** (12mg, 54 μmol) in DMSO/H₂O (1:3 V/V') into a nude mouse bearing a MatLu rat prostate tumor grown on the thigh (tumor size: 1.6cm $\times$ 1.4cm $\times$ 1.0cm), a broad, single ^{19}F peak was observed at $\delta_{\text{F}(8)}=15.96\text{ppm}$ ($\text{pH}_\text{e}=6.48$) with an acquisition time of 7 minutes (Figure 7).

Conclusion

Given the relevance of $\text{pH}_\text{i}/\text{pH}_\text{e}$ to tumor development and prognostic outcome, non-invasive techniques to sample cellular pH *in vivo* have great potential and are increasingly important in therapeutic oncology.^[1-3] As the most electronegative element, fluorine has played a key role in medicinal chemistry; the incorporation of fluorine and/or fluorine-containing groups into an organic molecule often drastically perturbs the properties of the parent compound.^[7,8] ^{19}F -MRS has been widely utilized in *in vivo* studies on drug absorption, distribution, metabolism and excretion due to its favorable MR properties, simplicity, and high sensitivity.^[1a,b] In this study, we have successfully synthesized two novel ^{19}F -NMR pH indicators **8** and **17**, and identified the following useful characteristics which make them well-suited for the *in vivo* assessment of pH using ^{19}F -MRS: (a) ideal pK_a (6.83~7.84); (b) sensitivity to pH (~0.40 ppm/pH unit); (c) ^{19}F -chemical shift response to pH ($\Delta\delta_{\text{F}}=1.38\sim1.61\text{ppm}$); (d) ^{19}F -signal enhancement, in which **8** was shown to be capable of *in vivo* sampling pH_e in various tumor models. Noting these features of **8** as a ^{19}F -MRS pH_e molecular probe, we believe it has promising potential in ^{19}F -MRI investigations of the tumor microenvironment for effective characterization of tumor heterogeneity with spatial and temporal resolution.

EXPERIMENTAL

General Methods

NMR spectra were recorded on a Varian Inova 400 spectrometer (400 MHz for ^1H , 100 MHz for ^{13}C , 376 MHz for ^{19}F , 121 MHz for ^{31}P). ^1H and ^{13}C chemical shifts are referenced to TMS as internal standard with CDCl_3 , or $\text{DMSO}-d_6$ as solvents; ^{19}F to a dilute solution of NaTFA in a capillary as external standard, chemical shifts are given in ppm. Mass spectra were obtained by positive and negative ESI-MS using a Micromass Q-TOF hybrid quadrupole/time-of-flight instrument (Micromass UK Ltd). Microanalyses were performed on a Perkin-Elmer 2400CHN microanalyser.

$\text{Hg}(\text{CN})_2$ was dried before use at 50°C for 1h, CH_2Cl_2 was dried over Drierite, acetonitrile was dried on CaH_2 and kept over molecular sieves under nitrogen. Solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated *in vacuo* below 45°C . 2, 3, 4, 6-tetra-*O*-acetyl- α -*D*-glucopyranosyl bromide was purchased from the Sigma Chemical Company. Column chromatography was performed on silica gel (200~300 mesh), and silica gel GF₂₅₄ used for TLC was purchased from the Aldrich Chemical Company. Detection was effected by spraying the plates with 5% ethanolic H_2SO_4 (followed by heating at 110°C for ~10 minutes) or by direct UV illumination of the plate. The purity of the final products was determined by HPLC with 95%.

6-Iodopyridoxine 2—To a solution of pyridoxine **1** (3.4 g, 20.0 mmol) in 10% K_2CO_3 aqueous solution (60 mL), iodine (5.04 g, 20.0 mmol) was added. The reaction mixture was vigorously stirred in the dark at room temperature for 2~3 h, after addition of Na_2SO_3 (320 mg), the reaction was quenched with concentrated HCl up to pH 3, then the precipitate was filtered and dried *in vacuo* over NaOH to give **2** (4.28 g, 73%) as a yellow powder, ^1H -NMR ($\text{DMSO}-d_6$), δ_{H} : 9.51 (1 H, s, HO-3), 5.82 (1 H, br, α^5 -OH), 5.15 (1H, br, α^4 -OH), 4.80 (2 H, d, J = 2.8 Hz, CH_2 -5), 4.57 (2 H, d, J = 3.2 Hz, CH_2 -4), 2.31 (3 H, s, CH_3 -2) ppm; ^{13}C -NMR ($\text{DMSO}-d_6$), δ_{C} : 150.43 (s, Py-C), 148.25 (s, Py-C), 136.21 (s, Py-C), 134.97 (s, Py-C), 112.11 (s, Py-C), 63.99 (s, CH_2 -5), 57.05 (s, CH_2 -4), 18.93 (s, CH_3 -2) ppm.

Anal. Calcd. for $\text{C}_8\text{H}_{10}\text{NO}_3\text{I}$ (%): C, 32.56, H, 3.42, N, 4.75; Found: C, 32.51, H, 3.39, N, 4.71.

3, α^4 , α^5 -Tri-*O*-acetyl-6-Iodopyridoxine 3—A solution of **2** (0.90 g, 3.0 mmol) in pyridine (20 mL) was treated with acetic anhydride (9 mL). After being stirred from 0°C to r.t. overnight, the mixture was co-evaporated with toluene under reduced pressure and the residue purified by flash silica gel column chromatography (eluent: 2:1 cyclohexane-EtOAc) to afford **3** (1.19 g, 93%) as white crystals, R_f 0.50 (3:2 cyclohexane-EtOAc), ^1H -NMR (CDCl_3), δ_{H} : 5.18 (2 H, s, CH_2 -5), 5.15 (2 H, s, CH_2 -4), 2.40 (3 H, s, CH_3 -2), 2.05, 2.03, 2.01 (9 H, 3s, $3\times\text{CH}_3\text{CO}$) ppm; ^{13}C -NMR (CDCl_3), δ_{C} : 173.97, 170.73, 168.43 (3s, $3\times\text{CH}_3\text{CO}$), 152.83 (s, Py-C), 150.33 (s, Py-C), 132.99 (s, Py-C), 129.83 (s, Py-C), 113.02 (s, Py-C), 66.33 (s, CH_2 -5), 58.76 (s, CH_2 -4), 20.94, 20.88, 19.63 (3s, $3\times\text{CH}_3\text{CO}$), 19.61 (s, CH_3 -2) ppm.

Anal. Calcd. for $\text{C}_{14}\text{H}_{16}\text{NO}_6\text{I}$ (%): C, 39.92, H, 3.83, N, 3.33; Found: C, 39.90, H, 3.80, N, 3.30.

3, α^4 , α^5 -Tri-*O*-Acetyl-6-Trifluoromethyl Pyridoxine 4—To a well stirred mixture of **3** (1.0 g, 2.4 mmol), CuI (456 mg, 2.4 mmol, 1.0 equiv.) and KF (168 mg, 2.9 mmol, 1.2 equiv.) in *N,N*-dimethylformamide (DMF, 5 mL) and *N*-methylpyrrolidine (NMP, 5 mL), Me_3SiCF_3 (537 μL , 2.9 mmol, 1.2 equiv.) was added under an argon atmosphere in the dark, then the reaction mixture was stirred at 80°C in a sealed glass pressure tube (15 mL) for 24h.

The mixture was diluted with CH₂Cl₂ (120 mL), filtered through Celite, washed with water, dried (Na₂SO₄) and concentrated *in vacuo*. The residue was purified on a silica gel column (2:1 cyclohexane-EtOAc) to yield **4** (0.35 g, 40%) as a syrup, R_f 0.37 (2:1 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H: 5.34 (2 H, s, CH₂-5), 5.16 (2 H, s, CH₂-4), 2.39 (3 H, s, CH₃-2), 2.38, 2.10, 2.02 (9 H, 3s, 3×CH₃CO) ppm; ¹³C-NMR (CDCl₃), δ_C: 170.54, 170.22, 168.41 (3s, 3×CH₃CO), 145.04 (s, Py-C_{2'}), 137.81 (s, Py-C_{3'}), 154.80 (s, Py-C_{4'}), 133.90 (q, ³J_{F-C} = 20.6 Hz, Py-C_{5'}), 140.27 (q, ²J_{F-C} = 32.6 Hz, Py-C_{6'}), 121.53 (q, ¹J_{F-C} = 272.6 Hz, CF₃), 66.27 (s, CH₂-5), 57.39 (s, CH₂-4), 20.89, 20.72, 20.68 (3s, 3×CH₃CO), 19.63 (s, CH₃-2) ppm.

Anal. Calcd. for C₁₅H₁₆NO₆F₃ (%): C, 49.59, H, 4.44, N, 3.86; Found: C, 49.55, H, 4.43, N, 3.84.

α⁴, α⁵-Di-O-Acetyl-6-Trifluoromethyl Pyridoxine 5—88 mg, 12% as a syrup, R_f 0.27 (2:1 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H: 5.24 (2 H, s, CH₂-5), 5.06 (2 H, s, CH₂-4), 2.33 (3 H, s, CH₃-2), 2.09, 2.02 (6 H, 2s, 2×CH₃CO) ppm; ¹³C-NMR (CDCl₃), δ_C: 170.12, 168.38 (2s, 2×CH₃CO), 144.84 (s, Py-C_{2'}), 137.41 (s, Py-C_{3'}), 154.77 (s, Py-C_{4'}), 133.30 (q, ³J_{F-C} = 18.6 Hz, Py-C_{5'}), 141.67 (q, ²J_{F-C} = 32.8 Hz, Py-C_{6'}), 121.13 (q, ¹J_{F-C} = 272.5 Hz, CF₃), 66.17 (s, CH₂-5), 57.29 (s, CH₂-4), 20.68, 20.57 (2s, 2×CH₃CO), 19.56 (s, CH₃-2) ppm.

Anal. Calcd. for C₁₃H₁₄NO₅F₃ (%): C, 48.60, H, 4.39, N, 4.36; Found: C, 48.55, H, 4.36, N, 4.32.

3, α⁴, α⁵-Tri-O-Benzyl-6-Iodopyridoxine 6—A solution of benzyl bromide (1.43 g, 8.0 mmol) in dry DMF (15 mL) was added dropwise over a period of 1~2h to a well stirred dry DMF (70 mL) solution of **2** (0.69 g, 2.4 mmol) and NaH (341 mg, 8.6 mmol, 60% dispersion in mineral oil), and the stirring continued for additional 4~5h, at the end of time TLC (4:1 cyclohexane-EtOAc) showed reaction complete, then MeOH (15 mL) was added slowly to react with the excess of the NaH. After most of the DMF was removed under reduced pressure at 55°C, the residue was dissolved in CH₂Cl₂ (125 mL) and washed with water, dried (Na₂SO₄), filtered and evaporated, the residue was purified by column chromatography on silica gel with 4:1 cyclohexane-EtOAc as the eluent to afford quantitatively **6** (1.32 g) as a syrup, R_f 0.56 (4:1 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H: 7.38 ~ 7.27 (15 H, m, Ph-H), 4.84 (2 H, s, PhCH₂-O₃), 4.64 (2 H, s, α⁴-OCH₂Ph), 4.56 (2 H, s, CH₂-4), 4.54 (2 H, s, CH₂-5), 4.41 (2 H, s, α⁵-OCH₂Ph), 2.48 (3 H, s, CH₃-2) ppm; ¹³C-NMR (CDCl₃), δ_C: 155.28 (s, Py-C), 152.76 (s, Py-C), 140.29 (s, Py-C), 136.24 (s, Py-C), 128.66 ~ 128.16 (m, Ph-C), 119.57 (s, Py-C), 76.97 (s, PhCH₂-O₃), 73.68 (s, α⁴-OCH₂Ph), 73.51 (s, α⁵-OCH₂Ph), 71.87 (s, CH₂-4), 63.24 (s, CH₂-5), 19.74 (s, CH₃-2) ppm.

Anal. Calcd. for C₂₉H₂₈NO₃I (%): C, 61.60, H, 4.99, N, 2.48; Found: C, 61.56, H, 4.96, N, 2.45.

3, α⁴, α⁵-Tri-O-Benzyl-6-Trifluoromethyl Pyridoxine 7—Trifluoromethylation of **6** (0.89 g, 1.6 mmol) with Me₃SiCF₃ (356 μL, 1.9 mmol, 1.2 equiv.) in the presence of CuI (304 mg, 1.6 mmol, 1.0 equiv.) and KF (110 mg, 1.9 mmol, 1.2 equiv.) in DMF-NMP (10 mL, 1:1 V/V') under an argon atmosphere in the dark, according to the procedures described for the preparation of **4**, furnished **3, α⁴, α⁵-tri-O-benzyl-6-trifluoromethyl pyridoxine 7** (0.78 g, 96%) as a syrup, R_f 0.64 (4:1 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H: 7.40 ~ 7.28 (15 H, m, Ph-H), 4.89 (2 H, s, PhCH₂-O₃), 4.65 (2 H, s, α⁴-OCH₂Ph), 4.59 (2 H, s, CH₂-4), 4.45 (2 H, s, CH₂-5), 4.40 (2 H, s, α⁵-OCH₂Ph), 2.54 (3 H, s, CH₃-2) ppm; ¹³C-NMR (CDCl₃), δ_C: 141.44 (s, Py-C_{2'}), 136.50 (s, Py-C_{3'}), 153.44 (s, Py-C_{4'}), 137.60

(q, $^3J_{F-C} = 21.4$ Hz, Py-C_{5'}), 142.07 (q, $^2J_{F-C} = 32.1$ Hz, Py-C_{6'}), 122.36 (q, $^1J_{F-C} = 273.9$ Hz, CF₃), 128.92 ~ 128.10 (m, Ph-C), 73.86 (s, PhCH₂-O₃), 73.70 (s, α⁴-OCH₂Ph), 64.08 (s, α⁵-OCH₂Ph), 64.05 (s, CH₂-4), 62.63 (s, CH₂-5), 20.01 (s, CH₃-2) ppm.

Anal. Calcd. for C₃₀H₂₈NO₃F₃ (%): C, 70.99, H, 5.56, N, 2.76; Found: C, 70.96, H, 5.54, N, 2.73.

6-Trifluoromethyl Pyridoxine 8—Hydrogenation (H₂, 30 psi) of **7** (0.70 g, 1.4 mmol) in anhydrous AcOH-EtOH (70 mL, 1:10 V/V') catalyzed by Pd/C (5%, 250 mg) for 2 days furnished the target molecule **8** (0.33 g, 100%) as crystals, R_f 0.34 (1:1 cyclohexane-EtOAc), ¹H-NMR (DMSO-*d*₆), δ_H: 4.88 (2 H, s, CH₂-5), 4.59 (2 H, s, CH₂-4), 4.50 (3 H, br, 3-OH, α⁴-OH, α⁵-OH), 2.40 (3 H, s, CH₃-2) ppm; ¹³C-NMR (DMSO-*d*₆), δ_C: 132.23 (s, Py-C_{2'}), 134.51 (s, Py-C_{3'}), 153.97 (s, Py-C_{4'}), 145.85 (s, Py-C_{5'}), 133.51 (q, $^2J_{F-C} = 31.3$ Hz, Py-C_{6'}), 122.94 (q, $^1J_{F-C} = 272.4$ Hz, CF₃), 56.55 (s, CH₂-4), 55.21 (s, CH₂-5), 19.27 (s, CH₃-2) ppm.

Anal. Calcd. for C₉H₁₀NO₃F₃ (%): C, 45.58, H, 4.25, N, 5.91; Found: C, 45.54, H, 4.23, N, 5.89.

α⁴, α⁵-Di-O-Benzyl-6-Trifluoromethyl Pyridoxine 9—0.58 g, 100%, syrup, R_f 0.52 (4:1 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H: 8.22 (1 H, s, 3-OH), 7.62 ~ 7.55 (10 H, m, Ph-H), 4.91 (2 H, s, α⁴-OCH₂Ph), 4.79 (2 H, s, CH₂-4), 4.73 (2 H, s, CH₂-5), 4.72 (2 H, s, α⁵-OCH₂Ph), 2.66 (3 H, s, CH₃-2) ppm; ¹³C-NMR (CDCl₃), δ_C: 138.12 (s, Py-C_{2'}), 138.17 (s, Py-C_{3'}), 149.47 (s, Py-C_{4'}), 147.26 (s, Py-C_{5'}), 140.02 (q, $^2J_{F-C} = 32.3$ Hz, Py-C_{6'}), 121.54 (q, $^1J_{F-C} = 273.2$ Hz, CF₃), 128.50 ~ 127.20 (m, Ph-C), 71.96 (s, α⁴-OCH₂Ph), 71.64 (s, α⁵-OCH₂Ph), 67.09 (s, CH₂-4), 62.58 (s, CH₂-5), 19.88 (s, CH₃-2) ppm.

Anal. Calcd. for C₂₃H₂₂NO₃F₃ (%): C, 66.18, H, 5.31, N, 3.36; Found: C, 66.13, H, 5.30, N, 3.34.

α⁴-O-Benzyl-6-Trifluoromethyl Pyridoxine 10—0.46 g, 100%, syrup, R_f 0.34 (4:1 cyclohexane-EtOAc), ¹H-NMR (DMSO-*d*₆), δ_H: 7.38 ~ 7.34 (5 H, m, Ph-H), 5.05 (2 H, s, α⁴-OCH₂Ph), 4.70 (2 H, br, 3-OH, α⁵-OH), 4.63 (2 H, s, CH₂-4), 4.54 (2 H, d, $J_{H-5,HO-5} = 6.2$ Hz, CH₂-5), 2.52 (3 H, s, CH₃-2) ppm; ¹³C-NMR (DMSO-*d*₆), δ_C: 132.03 (s, Py-C_{2'}), 137.30 (s, Py-C_{3'}), 153.76 (s, Py-C_{4'}), 147.93 (s, Py-C_{5'}), 136.58 (q, $^2J_{F-C} = 32.6$ Hz, Py-C_{6'}), 122.54 (q, $^1J_{F-C} = 272.8$ Hz, CF₃), 128.78 ~ 128.27 (m, Ph-C), 73.22 (s, α⁴-OCH₂Ph), 64.40 (s, CH₂-4), 61.07 (s, CH₂-5), 18.99 (s, CH₃-2) ppm.

Anal. Calcd. for C₁₆H₁₆NO₃F₃ (%): C, 58.71, H, 4.93, N, 4.28; Found: C, 58.68, H, 4.90, N, 4.27.

3-O-Benzyl-6-Iodopyridoxine 11—To a well stirred CH₂Cl₂-H₂O (20 mL, 1:1 V/V') bi-phasic mixture (pH 9~10) of **2** (1.0 g, 3.4 mmol) and TBAB (0.10 g, 0.31 mmol) as the phase-transfer catalyst, a solution of benzyl bromide (0.65 g, 3.73 mmol, 1.1 equiv.) in CH₂Cl₂ (10 mL) was added dropwise over a period of 4~5h at room temperature, and the stirring continued for an additional hour. The mixture was extracted with CH₂Cl₂ (4×20 mL), washed free of alkali, dried (Na₂SO₄), and concentrated, the residue was purified by column chromatography on silica gel with 3:2 cyclohexane-EtOAc as the eluent to afford major product **11** (1.31 g, 74%) as white crystalline, R_f 0.68 (1:2 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H: 7.41 ~ 7.37 (5 H, m, Ar-H), 4.92 (2 H, s, PhCH₂), 4.87 (2 H, d, $J_{H-4,HO-4} = 5.6$ Hz, CH₂-4), 4.73 (2 H, d, $J_{H-5,HO-5} = 6.4$ Hz, CH₂-5), 3.73 (2 H, br, α⁴-OH, α⁵-OH, exchangeable with D₂O), 2.50 (3 H, s, CH₃-2) ppm; ¹³C-NMR (CDCl₃), δ_C: 155.56 (s, Py-C), 152.03 (s, Py-C), 142.98 (s, Py-C), 136.22 (s, Py-C), 129.04 ~ 128.69 (m, Ph-C),

117.97 (s, Py-C), 76.93 (s, PhCH₂-O₃), 67.03 (s, CH₂-4), 57.44 (s, CH₂-5), 19.83 (s, CH₃-2) ppm.

Anal. Calcd. for C₁₅H₁₆NO₃I (%): C, 46.77, H, 4.19, N, 3.64; Found: C, 46.74, H, 4.17, N, 3.62.

3-O-Benzyl- α^4 , α^5 -Di-O-Allyl-6-Iodopyridoxine 12—To a well stirred dry DMF (80 mL) solution of **11** (1.20 g, 3.1 mmol) and NaH (0.50 g, 12.5 mmol, 60% dispersion in mineral oil), allyl bromide (1.13 g, 9.33 mmol) in dry DMF (10 mL) was added dropwise over a period of 1~2h, and the stirring continued for additional 4~5h, at the end of time TLC (4:1 cyclohexane-EtOAc) showed reaction complete, then MeOH (15 mL) was added slowly to react with the excess of the NaH. After most DMF was removed under reduced pressure at 55°C, the residue was dissolved in CH₂Cl₂ (150 mL) and washed with water, dried (Na₂SO₄), filtered and evaporated, the residue was purified by column chromatography on silica gel with 4:1 cyclohexane-EtOAc as the eluent to afford quantitatively **12** (1.45 g) as a syrup, R_f 0.58 (4:1 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H : 7.43 ~ 7.36 (5 H, m, Ar-H), 5.98 (1 H, dq, ³J_{1',2'} = 1.8 Hz, ³J_{2',3a'} = 20.0 Hz, ³J_{2',3b'} = 9.0 Hz, H-2'), 5.91 (1 H, dq, ³J_{1'',2''} = 1.8 Hz, ³J_{2'',3a''} = 22.4 Hz, ³J_{2'',3b''} = 9.0 Hz, H-2''), 5.35 (1 H, dt, ⁴J_{1',3'} = 1.0 Hz, ²J_{3a',3b'} = 2.4 Hz, H-3'), 5.26 (1 H, dt, ⁴J_{1'',3''} = 1.0 Hz, ²J_{3a'',3b''} = 2.4 Hz, H-3''), 4.89 (2 H, s, PhCH₂), 4.67 (2 H, s, CH₂-4), 4.60 (2 H, s, CH₂-5), 4.11 (2 H, dt, H-1'), 4.02 (2 H, dt, H-1''), 2.49 (3 H, s, CH₃-2) ppm; ¹³C-NMR (CDCl₃), δ_C : 155.27 (s, Py-C), 152.84 (s, Py-C), 140.40 (s, Py-C), 136.65 (s, Py-C), 134.63 (s, α^4 -OCH₂CH=CH₂), 134.26 (s, α^5 -OCH₂CH=CH₂), 128.89 ~ 128.14 (m, Ph-C), 119.45 (s, Py-C), 118.25 (s, α^4 -OCH₂CH=CH₂), 118.03 (s, α^5 -OCH₂CH=CH₂), 76.97 (s, PhCH₂-O₃), 72.46 (s, α^4 -OCH₂CH=CH₂), 72.29 (s, α^5 -OCH₂CH=CH₂), 71.96 (s, CH₂-4), 63.29 (s, CH₂-5), 19.76 (s, CH₃-2) ppm.

Anal. Calcd. for C₂₁H₂₄NO₃I (%): C, 54.20, H, 5.20, N, 3.01; Found: C, 54.16, H, 5.18, N, 3.00.

3-O-Benzyl- α^4 , α^5 -Di-O-Allyl-6-Trifluoromethyl Pyridoxine 13—

Trifluoromethylation of **12** (1.10 g, 2.4 mmol) with Me₃SiCF₃ (537 μ L, 2.9 mmol, 1.2 equiv.) in the presence of CuI (456 mg, 2.4 mmol, 1.0 equiv.) and KF (168 mg, 2.9 mmol, 1.2 equiv.) in DMF-NMP (10 mL, 1:1 V/V') under an argon atmosphere in the dark, according to the procedures described for the preparation of **4** and **7**, yielded **13** (0.90 g, 92%) as a syrup, R_f 0.71 (3:1 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H : 7.45 ~ 7.34 (5 H, m, Ar-H), 5.97 (1 H, dq, ³J_{1',2'} = 4.8 Hz, ³J_{2',3a'} = 20.8 Hz, ³J_{2',3b'} = 9.2 Hz, H-2'), 5.91 (1 H, dq, ³J_{1'',2''} = 1.8 Hz, ³J_{2'',3a''} = 22.0 Hz, ³J_{2'',3b''} = 9.0 Hz, H-2''), 5.32 (1 H, dt, ⁴J_{1',3'} = 1.2 Hz, ²J_{3a',3b'} = 2.6 Hz, H-3'), 5.23 (1 H, dt, ⁴J_{1'',3''} = 0.8 Hz, ²J_{3a'',3b''} = 2.2 Hz, H-3''), 4.96 (2 H, s, PhCH₂), 4.71 (2 H, s, CH₂-4), 4.61 (2 H, s, CH₂-5), 4.10 (2 H, dt, H-1'), 4.05 (2 H, dt, H-1''), 2.58 (3 H, s, CH₃-2) ppm; ¹³C-NMR (CDCl₃), δ_C : 136.50 (s, Py-C₂), 136.12 (s, Py-C₃), 153.32 (s, Py-C₄), 141.73 (q, ³J_{F-C} = 6.8 Hz, Py-C₅), 134.94 (q, ²J_{F-C} = 32.4 Hz, Py-C₆), 122.31 (q, ¹J_{F-C} = 273.9 Hz, CF₃), 133.38 (s, α^4 -OCH₂CH=CH₂), 131.18 (s, α^5 -OCH₂CH=CH₂), 129.64 ~ 127.45 (m, Ph-C), 118.24 (s, α^4 -OCH₂CH=CH₂), 118.21 (s, α^5 -OCH₂CH=CH₂), 78.41 (s, PhCH₂-O₃), 72.69 (s, α^4 -OCH₂CH=CH₂), 72.39 (s, α^5 -OCH₂CH=CH₂), 71.21 (s, CH₂-4), 64.04 (s, CH₂-5), 20.59 (s, CH₃-2) ppm.

Anal. Calcd. for C₂₂H₂₄NO₃F₃ (%): C, 64.86, H, 5.94, N, 3.44; Found: C, 64.82, H, 5.91, N, 3.42.

3-O-Benzyl- α^4 , α^5 -Di-O-(3-Hydroxypropyl)-6-Trifluoromethyl Pyridoxine 14—To a solution of **13** (0.41 g, 1.0 mmol) in dry dioxane (10 mL) was added 9-BBN (8 mL, 4 mmol, 0.5 M solution in THF) dropwise at 0°C under argon. The reaction mixture was

stirred at r.t. for 24h, cooled to 0°C, aqueous NaOH (3 M, 8 mL) and 30% H₂O₂ (1.3 mL) were added. The reaction mixture was stirred at r.t. for 2 days, the aqueous phase was extracted with ethyl acetate (4×50 mL). The combined organic phases were washed with saturated NaCl solution, and dried (Na₂SO₄). The solution was filtered and the filtrate concentrated *in vacuo* to give an almost colorless syrup, which was purified by column chromatography on silica gel with 1:3 cyclohexane-EtOAc as the eluent to afford **14** (0.38 g, 86%) as a syrup, *R*_f 0.35 (1:4 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H: 7.42 ~ 7.36 (5 H, m, Ar-H), 4.93 (2 H, s, PhCH₂), 4.86 (2 H, s, CH₂-4), 4.62 (2 H, s, CH₂-5), 3.74 ~ 3.60 (8 H, m, α⁴-OCH₂CH₂CH₂OH, α⁵-OCH₂CH₂CH₂OH), 2.55 (3 H, s, CH₃-2), 2.92 (2H, br, α⁴-O(CH₂)₃OH, α⁵-O(CH₂)₃OH), 1.86 ~ 1.76 (4 H, m, α⁴-OCH₂CH₂CH₂OH, α⁵-OCH₂CH₂CH₂OH) ppm; ¹³C-NMR (CDCl₃), δ_C: 135.70 (s, Py-C₂'), 130.81 (s, Py-C₃'), 153.36 (s, Py-C₄'), 136.34 (q, ³*J*_{F-C} = 8.3 Hz, Py-C₅'), 141.59 (q, ²*J*_{F-C} = 32.0 Hz, Py-C₆'), 122.22 (q, ¹*J*_{F-C} = 273.9 Hz, CF₃), 129.00 ~ 127.84 (m, Ph-C), 76.85 (s, PhCH₂-O₃), 72.66 (s, CH₂-4), 69.57, 69.56 (2s, α⁴-OCH₂(CH₂)₂OH, α⁵-OCH₂(CH₂)₂OH), 63.72 (s, CH₂-5), 60.79, 60.63 (2s, α⁴-O(CH₂)₂CH₂OH, α⁵-O(CH₂)₂CH₂OH), 32.36, 32.27 (2s, α⁴-OCH₂CH₂CH₂OH, α⁵-OCH₂CH₂CH₂OH), 19.78 (s, CH₃-2) ppm.

Anal. Calcd. for C₂₂H₂₈NO₅F₃ (%): C, 59.59, H, 6.36, N, 3.16; Found: C, 59.56, H, 6.34, N, 3.14.

3-O-Benzyl-α⁴, α⁵-Di-O-[3'-O-(2, 3, 4, 6-Tetra-O-Acetyl-β-D-Glucopyranosyl)-Propyl]-6-Trifluoromethyl Pyridoxine 15—A solution of 2, 3, 4, 6-tetra-O-acetyl-α-D-glucopyranosyl bromide (0.75 g, 1.45 mmol, 1.2 equiv.) in anhydrous CH₂Cl₂ (5 mL) was added dropwise into the solution of **14** (0.34 g, 0.75 mmol) and Hg(CN)₂ (0.52 g, 1.21 mmol) as a promoter in dry acetonitrile (10 mL) containing powdered molecular sieves (4 Å, 1.3 g) with vigorous stirring at r.t. under an argon atmosphere in the dark for 12h. The mixture was diluted with CH₂Cl₂ (60 mL), filtered through Celite, washed with water, dried (Na₂SO₄) and concentrated *in vacuo*. The residue was purified on a silica gel column (1:1 cyclohexane-EtOAc) to yield the title compound **15** (0.73 g, 88%) as a syrup, *R*_f 0.50 (1:1 cyclohexane-EtOAc), ¹H-NMR (CDCl₃), δ_H: 7.45 ~ 7.39 (5 H, m, Ar-H), 4.94 (2 H, s, PhCH₂), 4.68 (2 H, s, CH₂-4), 4.64 (2 H, s, CH₂-5), 4.15 ~ 4.09 (4 H, m, α⁴-OCH₂(CH₂)₂O-, α⁵-OCH₂(CH₂)₂O-), 3.62 ~ 3.54 (4 H, m, α⁴-O(CH₂)₂CH₂O-, α⁵-O(CH₂)₂CH₂O-), 2.57 (3 H, s, CH₃-2), 1.94 ~ 1.84 (4 H, m, α⁴-OCH₂CH₂CH₂O-, α⁵-OCH₂CH₂CH₂O-), 4.48 (1 H, d, *J*_{1',2'} = 8.0 Hz, H-1'), 4.41 (1 H, d, *J*_{1'',2''} = 7.6 Hz, H-1''), 5.00 (1 H, dd, *J*_{2',3'} = 10.0 Hz, H-2'), 4.96 (1 H, dd, *J*_{2'',3''} = 9.8 Hz, H-2''), 5.20 (1 H, dd, *J*_{3',4'} = 3.2 Hz, H-3'), 5.17 (1 H, dd, *J*_{3'',4''} = 3.6 Hz, H-3''), 5.10 (1 H, dd, *J*_{4',5'} = 5.6 Hz, H-4'), 5.04 (1 H, dd, *J*_{4'',5''} = 5.4 Hz, H-4''), 3.70 (1 H, m, H-5'), 3.68 (1 H, m, H-5''), 4.26 (1 H, dd, *J*_{5',6a'} = 4.8 Hz, *J*_{6a',6b'} = 13.6 Hz, H-6a'), 4.23 (1 H, dd, *J*_{5'',6a''} = 4.4 Hz, *J*_{6a'',6b''} = 12.4 Hz, H-6a''), 3.93 (1 H, dd, *J*_{5',6b'} = 5.6 Hz, H-6b'), 3.90 (1 H, dd, *J*_{5'',6b''} = 4.8 Hz, H-6b''), 2.06 ~ 1.99 (24 H, 8s, 8×CH₃CO) ppm; ¹³C-NMR (CDCl₃), δ_C: 171.23 ~ 169.37 (8s, 8×CH₃CO), 136.37 (s, Py-C₂'), 131.00 (s, Py-C₃'), 153.41 (s, Py-C₄'), 141.18 (q, ³*J*_{F-C} = 11.5 Hz, Py-C₅'), 141.82 (q, ²*J*_{F-C} = 32.8 Hz, Py-C₆'), 122.27 (q, ¹*J*_{F-C} = 274.0 Hz, CF₃), 129.00 ~ 127.88 (m, Ph-C), 76.88 (s, PhCH₂-O₃), 71.49 (s, CH₂-4), 68.14 (s, α⁴-OCH₂(CH₂)₂O-), 68.02 (s, α⁵-OCH₂(CH₂)₂O-), 67.07 (s, α⁴-O(CH₂)₂CH₂O-), 67.01 (s, α⁵-O(CH₂)₂CH₂O-), 63.52 (s, CH₂-5), 30.00 (s, α⁴-OCH₂CH₂CH₂O-), 29.13 (s, α⁵-OCH₂CH₂CH₂O-), 20.10 (s, CH₃-2), 101.02 (s, C-1', C-1''), 68.58 (s, C-2'), 68.51 (s, C-2''), 71.95 (s, C-3'), 71.87 (s, C-3''), 67.76 (s, C-4'), 67.66 (s, C-4''), 73.01 (s, C-5'), 72.92 (s, C-5''), 61.60 (s, C-6'), 61.50 (s, C-6''), 21.14 ~ 20.63 (8s, 8×CH₃CO) ppm. ESIMS: *m/z* 1103 [M⁺] (40%), 1104 [M+1] (28%).

Anal. Calcd. for C₅₀H₆₄NO₂₃F₃ (%): C, 54.40, H, 5.84, N, 1.27; Found: C, 54.36, H, 5.83, N, 1.25.

3-O-Benzyl- α^4 , α^5 -Di-O-[3'-O-(β -D-Glucopyranosyl)-Propyl]-6-Trifluoromethyl Pyridoxine 16—A solution of **15** (0.70 g) in anhydrous MeOH (20 mL) containing 0.5 M NH_3 was vigorously stirred from 0°C to rt for 2 days until TLC showed reaction complete, evaporated to dryness *in vacuo*. Chromatography of the crude syrup on silica gel with EtOAc-MeOH (4:1) afforded **16** (0.49 g) as a syrup in quantitative yield, R_f 0.36 (1:4 MeOH-EtOAc), $^1\text{H-NMR}$ (CDCl_3), δ_{H} : 7.52 ~ 7.39 (5 H, m, Ar-H), 5.00 (2 H, s, PhCH_2), 3.60 (2 H, s, CH_2 -4), 3.49 (2 H, s, CH_2 -5), 3.69 ~ 3.63 (4 H, m, $\alpha^4\text{-OCH}_2(\text{CH}_2)_2\text{O-}$, $\alpha^5\text{-OCH}_2(\text{CH}_2)_2\text{O-}$), 3.47 ~ 3.37 (4 H, m, $\alpha^4\text{-O}(\text{CH}_2)_2\text{CH}_2\text{O-}$, $\alpha^5\text{-O}(\text{CH}_2)_2\text{CH}_2\text{O-}$), 2.52 (3 H, s, CH_3 -2), 1.83 ~ 1.78 (4 H, m, $\alpha^4\text{-OCH}_2\text{CH}_2\text{CH}_2\text{O-}$, $\alpha^5\text{-OCH}_2\text{CH}_2\text{CH}_2\text{O-}$), 4.29 (2 H, d, $J_{\text{H-2,OH-2}} = 7.6$ Hz, HO-2', 2''), 4.61 (1 H, d, $J_{\text{H-3',OH-3'}} = 5.2$ Hz, HO-3'), 4.54 (1 H, d, $J_{\text{H-3',OH-3'}} = 5.2$ Hz, HO-3''), 4.95 (1 H, d, $J_{\text{H-4',OH-4'}} = 4.6$ Hz, HO-4'), 4.91 (1 H, d, $J_{\text{H-4',OH-4'}} = 4.4$ Hz, HO-4''), 4.44 (2 H, br, HO-6', 6''), 4.11 (1 H, d, $J_{1'',2''} = 8.0$ Hz, H-1'), 4.08 (1 H, d, $J_{1'',2''} = 7.6$ Hz, H-1''), 2.96 (1 H, dd, $J_{2',3'} = 10.1$ Hz, H-2'), 2.91 (1 H, dd, $J_{2'',3''} = 9.6$ Hz, H-2''), 3.08 (1 H, dd, $J_{3',4'} = 3.0$ Hz, H-3'), 3.05 (1 H, dd, $J_{3'',4''} = 3.4$ Hz, H-3''), 3.84 (1 H, dd, $J_{4',5'} = 6.4$ Hz, H-4'), 3.80 (1 H, dd, $J_{4'',5''} = 6.4$ Hz, H-4''), 3.47 (1 H, m, H-5'), 3.43 (1 H, m, H-5''), 3.67 (1 H, dd, $J_{5',6a'} = 4.6$ Hz, $J_{6a',6b'} = 11.2$ Hz, H-6a'), 3.64 (1 H, dd, $J_{5'',6a''} = 4.8$ Hz, $J_{6a'',6b''} = 9.6$ Hz, H-6a''), 3.58 (1 H, dd, $J_{5',6b'} = 5.2$ Hz, H-6b'), 3.54 (1 H, dd, $J_{5'',6b''} = 4.4$ Hz, H-6b'') ppm; $^{13}\text{C-NMR}$ (CDCl_3), δ_{C} : 136.50 (s, Py-C_{2'}), 131.27 (s, Py-C_{3'}), 153.16 (s, Py-C_{4'}), 141.36 (q, $^3J_{\text{F-C}} = 16.0$ Hz, Py-C_{5'}), 140.25 (q, $^2J_{\text{F-C}} = 32.1$ Hz, Py-C_{6'}), 122.32 (q, $^1J_{\text{F-C}} = 273.9$ Hz, CF₃), 128.80 ~ 128.53 (m, Ph-C), 76.88 (s, $\text{PhCH}_2\text{-O}_3$), 72.07 (s, CH_2 -4), 68.04 (s, $\alpha^4\text{-OCH}_2(\text{CH}_2)_2\text{O-}$), 67.82 (s, $\alpha^5\text{-OCH}_2(\text{CH}_2)_2\text{O-}$), 66.00 (s, $\alpha^4\text{-O}(\text{CH}_2)_2\text{CH}_2\text{O-}$), 65.89 (s, $\alpha^5\text{-O}(\text{CH}_2)_2\text{CH}_2\text{O-}$), 63.98 (s, CH_2 -5), 32.66 (s, $\alpha^4\text{-OCH}_2\text{CH}_2\text{CH}_2\text{O-}$), 29.76 (s, $\alpha^5\text{-OCH}_2\text{CH}_2\text{CH}_2\text{O-}$), 22.57 (s, CH_3 -2), 103.11 (s, C-1'), 103.04 (s, C-1''), 68.18 (s, C-2'), 67.96 (s, C-2''), 70.38 (s, C-3'), 70.15 (s, C-3''), 65.98 (s, C-4'), 65.89 (s, C-4''), 73.52 (s, C-5'), 73.15 (s, C-5''), 61.30 (s, C-6'), 61.16 (s, C-6'') ppm. ESIMS: m/z 767 [M^+] (30%), 768 [$\text{M}+1$] (18%).

Anal. Calcd. for $\text{C}_{34}\text{H}_{48}\text{NO}_{15}\text{F}_3$ (%): C, 53.19, H, 6.30, N, 1.82; Found: C, 53.14, H, 6.28, N, 1.80.

α^4 , α^5 -Di-O-[3'-O-(β -D-Glucopyranosyl)-Propyl]-6-Trifluoromethyl Pyridoxine 17—Hydrogenation (H_2 , 30 psi) of **16** (0.45 g) in anhydrous EtOH (30 mL) catalyzed by Pd/C (5%, 125 mg) for 1 day furnished the target molecule **17** (0.40 g, 100%) as a syrup, R_f 0.30 (1:2 MeOH-EtOAc), $^1\text{H-NMR}$ ($\text{DMSO}-d_6$), δ_{H} : 4.78 (2 H, s, CH_2 -4), 4.54 (2 H, s, CH_2 -5), 4.94 ~ 4.85 (4 H, m, $\alpha^4\text{-OCH}_2(\text{CH}_2)_2\text{O-}$, $\alpha^5\text{-OCH}_2(\text{CH}_2)_2\text{O-}$), 3.40 ~ 3.15 (4 H, m, $\alpha^4\text{-O}(\text{CH}_2)_2\text{CH}_2\text{O-}$, $\alpha^5\text{-O}(\text{CH}_2)_2\text{CH}_2\text{O-}$), 2.38 (3 H, s, CH_3 -2), 1.89 ~ 1.80 (4 H, m, $\alpha^4\text{-OCH}_2\text{CH}_2\text{CH}_2\text{O-}$, $\alpha^5\text{-OCH}_2\text{CH}_2\text{CH}_2\text{O-}$), 4.40 (1 H, d, $J_{1',2'} = 7.6$ Hz, H-1'), 4.07 (1 H, d, $J_{1'',2''} = 8.8$ Hz, H-1''), 3.06 (1 H, dd, $J_{2',3'} = 10.0$ Hz, H-2'), 3.02 (1 H, dd, $J_{2'',3''} = 9.6$ Hz, H-2''), 3.18 (1 H, dd, $J_{3',4'} = 2.8$ Hz, H-3'), 3.10 (1 H, dd, $J_{3'',4''} = 3.4$ Hz, H-3''), 3.85 (1 H, dd, $J_{4',5'} = 5.4$ Hz, H-4'), 3.81 (1 H, dd, $J_{4'',5''} = 5.6$ Hz, H-4''), 3.57 (1 H, m, H-5'), 3.54 (1 H, m, H-5''), 3.77 (1 H, dd, $J_{5',6a'} = 4.6$ Hz, $J_{6a',6b'} = 11.4$ Hz, H-6a'), 3.62 (1 H, dd, $J_{5'',6a''} = 4.4$ Hz, $J_{6a'',6b''} = 9.8$ Hz, H-6a''), 3.56 (1 H, dd, $J_{5',6b'} = 5.0$ Hz, H-6b'), 3.52 (1 H, dd, $J_{5'',6b''} = 4.4$ Hz, H-6b'') ppm; $^{13}\text{C-NMR}$ ($\text{DMSO}-d_6$), δ_{C} : 148.05 (s, Py-C_{2'}), 132.81 (s, Py-C_{3'}), 154.84 (s, Py-C_{4'}), 130.25 (q, $^3J_{\text{F-C}} = 12.6$ Hz, Py-C_{5'}), 135.45 (q, $^2J_{\text{F-C}} = 32.1$ Hz, Py-C_{6'}), 123.71 (q, $^1J_{\text{F-C}} = 273.6$ Hz, CF₃), 78.15 (s, CH_2 -4), 74.37 (s, $\alpha^4\text{-OCH}_2(\text{CH}_2)_2\text{O-}$), 74.14 (s, $\alpha^5\text{-OCH}_2(\text{CH}_2)_2\text{O-}$), 71.13 (s, $\alpha^4\text{-O}(\text{CH}_2)_2\text{CH}_2\text{O-}$), 71.08 (s, $\alpha^5\text{-O}(\text{CH}_2)_2\text{CH}_2\text{O-}$), 77.27 (s, CH_2 -5), 30.92 (s, $\alpha^4\text{-OCH}_2\text{CH}_2\text{CH}_2\text{O-}$), 29.44 (s, $\alpha^5\text{-OCH}_2\text{CH}_2\text{CH}_2\text{O-}$), 21.48 (s, CH_3 -2), 103.73 (s, C-1', C-1''), 68.97 (s, C-2'), 68.55 (s, C-2''), 70.98 (s, C-3'), 70.89 (s, C-3''), 67.23 (s, C-4'), 67.18 (s, C-4''), 73.09 (s, C-5'), 72.29 (s, C-5''), 62.17 (s, C-6'), 62.02 (s, C-6'') ppm. ESIMS: m/z 677 [M^+] (25%), 678 [$\text{M}+1$] (12%).

Anal. Calcd. for $C_{27}H_{42}NO_{15}F_3$ (%): C, 47.86, H, 6.25, N, 2.07; Found: C, 47.81, H, 6.23, N, 2.04.

^{19}F -NMR

The ^{19}F -NMR data versus pH were measured in NMR tubes using a combination pH electrode (Wilmaad, Buena, NJ) attached to a pH meter (Corning 220, Sudbury, UK), and for titration curves the pH was altered by addition of NaOH or HCl aq. Solutions. *In vivo* ^{19}F -NMR data were acquired using a 4.7T horizontal bore magnet with a Varian INOVA Unity system (Palo Alto, CA, USA, 188 MHz ^{19}F).

Blood

Fresh whole blood was drawn from the lateral ear of New Zealand white rabbits and stored chilled in the presence of heparin prior to ^{19}F -NMR studies.

Heart Perfusion

Langendorff retrograde perfusion was performed with recycled phosphate-free, modified Kres-Henseleit buffer oxygenated with carbogen at 37°C under a pressure of 100cm H₂O, as described in detail previously.^[4–6]

Animal Studies

Animal studies were performed in accordance with protocols approved by the UT Southwestern Institutional Animal Care and Use Committee. Dunning prostate tumor R3327-AT1, rat mammary tumor 13762NF cells were respectively implanted in a skin pedicle on the fore-back of a Copenhagen, Fisher 344 rat, and Dunning prostate tumor Mat-Lu cells were implanted subcutaneously in thighs of nude mice. Anesthesia was induced in an induction chamber with isoflurane and maintained during the surgery with a nose cone at 1.3% isoflurane/air (1.0 dm³/min). Once the tumor had grown to the requisite sizes, animals were anesthetized (isoflurane/air), injected i.p. with a solution of pH indicator **8** and NaTFA, then placed on a platform with a 2 cm diameter home built volume coil around the tumor. The animal-bed was inserted into the bore of the MR scanner and ^{19}F -NMR spectra were acquired immediately after tuning the coil to the ^{19}F resonance frequency. Animal temperature was maintained at 37°C by a warm pad with circulating water during acquisition.

Tumor Micro-Electrode Studies

Measurement of pHe was accomplished with the 20G combination needle electrode (Model 818, Diamond General, Ann Arbor, MI) and digital pH meter (Model 820A, Orion Research). Spatial pHe was mapped in three 13762NF breast tumor bearing female Fisher 344 rats. After anesthetizing the animal, the tumor was gently clamped into a fixed position. The pH electrode was clamped to a micro-caliper insertion device and inserted along the central track of the central plane of the tumor, until the needle reached the opposing side of the tumor wall. The pH needle was withdrawn in 0.5mm steps, and allowed to stabilize for 2 minutes prior to measurement. The pH response was recorded at each step, and the electrode was stepped backwards until withdrawn. This process was repeated along two additional parallel tracks, 0.5 cm anterior and 0.5 cm posterior to the central track.

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ABBREVIATIONS

FPOL	6-Fluoropyridoxine
CF₃	Trifluoromethyl
Me₃SiCF₃	(Trifluoromethyl)trimethylsilane
9-BBN	9-Borabicyclo-[3.3.1]nonane
NaTFA	Sodium trifluoroacetate
pHe	Extracellular pH value
pHi	Intracellular pH value
DMSO	Dimethyl sulfoxide
MRS	Magnetic resonance spectroscopy
NMR	Nuclear magnetic resonance
MRI	Magnetic resonance imaging
TLC	Thin layer chromatography
DMF	<i>N, N</i> -dimethylformamide
NMP	<i>N</i> -methylpyrrolidine.

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Figure 1.
The structure of 6-fluoropyridoxine (FPOL).

Figure 2. Reagents and conditions

(a) I_2 , 10% K_2CO_3 , r.t. 2~3h, in the dark; Na_2SO_3 ; HCl , 73%; (b) Ac_2O -Pyridine, $0^\circ\text{C} \rightarrow \text{r.t.}$, 24h, 93% ($\rightarrow$ **3**); (c) NaH , benzyl bromide (3.3 equiv.), DMF, r.t., 5~7h, 100% ($\rightarrow$ **6**); (d) Me_3SiCF_3 (1.2 equiv.), CuI (1.0 equiv.), KF (1.2 equiv.), Ar, DMF-NMP (1:1 V/V'), 80°C , 24hr, 40% ($\rightarrow$ **4**) or 96% ($\rightarrow$ **7**); (e) NH_3 -MeOH, $0^\circ\text{C} \rightarrow \text{r.t.}$, 24h, quantitative yield ($\rightarrow$ **8**); (f) H_2 (30psi), AcOH/EtOH (1:10 V/V'), Pd/C (5% W/W'), r.t., 2 days, quantitative yield ($\rightarrow$ **8**).

Figure 3. Reagents and conditions

(a) Benzyl bromide (1.1 equiv.), CH_2Cl_2 - H_2O , pH 9~10, r.t., TBAB, 4~5h, 74%; (b) NaH, allyl bromide (1.5 equiv.), DMF, r.t., 4h, 100%; (c) Me_3SiCF_3 (1.2 equiv.), CuI (1.0 equiv.), KF (1.2 equiv.), Ar, DMF-NMP (1:1 V/V'), 80°C, 24h, 92%; (d) 9-BBN (2.0 equiv.), Ar, dioxane, 0°C, 24h; NaOH, H_2O_2 , r.t., 48h, 86%; (e) 2, 3, 4, 6-tetra-*O*-acetyl- α -*D*-glucopyranosyl bromide (1.2 equiv.), $\text{Hg}(\text{CN})_2$ (1.5 equiv.), 4Å M.S., CH_2Cl_2 , r.t., 12h, 88%; (f) NH_3 -MeOH, 0°C→r.t., 48h, quantitative yield; (g) 30 psi H_2 , EtOH, Pd/C (5% W/W'), r.t., 12h, quantitative yield.

Figure 4.
The titration curves of **8** and **17** in saline at 25°C.

Figure 5.
 ^{19}F -NMR spectra (376 MHz, 25°C) of **8** in (a) Langendorff perfused rat heart, $\delta_{\text{F}} = 16.41$ ppm ($\text{pH}_{\text{e}} = 7.39$); (b) whole rabbit blood, $\delta_{\text{F}} = 16.52$ ppm ($\text{pH}_{\text{e}} = 7.44$).

Figure 6.

(A) *In vivo* ^{19}F -NMR spectrum (188MHz, 37°C) of **8** (320mg/kg) in Fisher 344 rat bearing pedicle 13762NF breast tumor, i.p. injection, tumor size: 1.8cm×1.0cm×2.0cm, $\delta_{\text{F}}=16.23\text{ppm}$ corresponding to $\text{pH}_{\text{e}}=7.14$, acquisition time: 8 minutes. (B) Distribution of pH_{e} values measured in a group of three Fisher 344 rats bearing 13762NF breast tumors using needle electrode: mean value $\text{pH}=7.03$ (arrow).

Figure 7.
In vivo ^{19}F -NMR spectrum (188MHz, 37°C) of **8** (12mg) in nude mouse bearing MatLu rat prostate tumor on thigh, i.p. injection, tumor size: 1.6cm×1.4cm×1.0cm, $\delta_{\text{F}(\text{8})}$ =15.96ppm corresponding to pH_e =6.48, acquisition time: 7 minutes.

Scheme 1.

The proposed reaction mechanism for the formation of **5**.

Table 1

Acidities and ^{19}F -NMR/pH properties of **8** and **17** at 25°C.

Compd.	pK_a	^{19}F NMR			
		$\delta_{(\text{acid})}$	$\delta_{(\text{base})}$	$\Delta\delta$	ppm/pH unit
8	6.83	15.15	16.76	1.61	0.40
17	7.84	15.28	16.66	1.38	0.43
					pH range
					5.40~9.40
					6.75~9.93